In re Application of: Sah, et al.

Application No.: 10/813,203

Filed: March 29, 2004

Page 2

PATENT ATTY. DOCKET NO.: REGEN1610-1

AMENDMENT

Amendments to the Specification:

Following the abstract, please insert the attached Sequence Listing with subsequent page numbering thereafter.

Please replace full paragraph at page 34, line 9 bridging to page 35 line 18 with the following amended paragraph:

The levels of mRNA encoding glutamate receptor subunits were also evaluated for clone B4 via PCR. For proliferating conditions, clone B4 at passage 15 or 24 was grown with FGF-2, EGF, PDGF and conditioned medium, as described above, for 3 days. Differentiation was achieved by growth of clone B4 (again at passage 15 or 24) for 8 days in tetracycline, high K⁺, NT-3 and BDNF, as described above. Human fetal brain tissue (200 mg) or cell pellets (~0.5 to 4 x 10⁶ cells) were snapfrozen and stored at -80°C until mRNA isolation. PolyA⁺ RNA was prepared using the Micro-FastTrack Kit (Pharmacia Biothech, Piscataway, NJ). PCR primers were designed using human sequences obtained from the GenBank database, and Oligo software. For PCR amplification, 15 ng of cDNA template was incubated with the oligonucleotide pair and 2.5 units of Taq polymerase (Perkin-Elmer, Foster City, CA) in 100 μL of assay buffer containing 100 mM Tris-HCI, pH 8.3; 500 mM KCl. Cycle parameters were: 5 min at 94°C, 5 min at 55°C, 1 min at 72°C, 1 min at 94°C, 1 min at 55°C, followed by 7 min at 72°C for 35 cycles. The resultant products were resolved on 1.2% agarose gels and stained with ethidium bromide for visualization. PCR conditions were optimized using human fetal brain cDNA as a positive control and cDNA libraries from Hela (GluR1-7), CHO (adenosine A₂, mGlu5b and 5-HT1A), 3T3 (5-HT2a) or HEK293 (GABAR α1) cells as negative controls. If a prominent band of the correct size was present in the positive control, then the following additional controls were carried out to verify that the PCR product was gene-specific: reverse transcriptase omission during cDNA preparation, Rnase addition during mRNA preparation, PCR with each primer alone, and sequence-specific probe hybridization. Primer sets used were:

NMDAR1 5' primer: 5'-AACCTGCAGAACCGCAAG-3' (1063-1080) (SEQ ID NO:1)

3' primer: 3'-GCTTGATGAGCAGGTCTATGC-3' (1376-1396) (SEQ ID NO:2)

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Page 3

GluR1	5' primer: 5'-AGATTTGCTTTGTGGCAA-3' (230-247) (SEQ ID NO:3)
	3' primer: 5'-ATTCTCCAGGTCCTGAAA-3' (668-685) (SEQ ID NO:4)
CluR2	5' primer: 5'-CGGAAGATTGGCTACTGG-3' (1310-1327) (SEQ ID NO:5)
	3' primer: 5'-TTAGCCGTGTAGGAGGAG-3' (2062-2080) (SEQ ID NO:6)
GluR3	5' primer: 5'-GACACACGACGCAATACTGG-3' (980-999) (SEQ ID NO:7)
	3' primer: 5'-TGAGAATACGCCTGGTTTTG-3' (1672-1691) (SEQ ID NO:8)
GluR4	5' primer: 5'-TGGTACGAGAGGAGGTCATT-3' (1514-1534) (SEQ ID NO:9)
	3' primer: 5'-TCTGGCTTTGTTTCTTATGG-3' (2561-2580) (SEQ ID NO:10)
GluR5	5' primer: 5'-CAAAGACAAGTCCAGCAA-3' (1254-1271) (SEQ ID NO:11)
	3' primer: 5'-CCAACTCCAAACCAGAAA-3' (1833-1850) (SEQ ID NO:12)
GluR6	5' primer: 5'-TTTGCTGGATGGATTTATG-3' (930-949) (SEQ ID NO:13)
	3' primer: 5'-AAAGAACGATTGGATAAGG-3' (1280-1298) (SEQ ID NO:14)
GluR7	5' primer: 5'- GCGTCTTCTCCTCCAATC-3' (1676-1696) (SEQ ID NO:15)
	3' primer: 5'- ATGCCCTCCTCGTTGTTCTTC-3' (2175-2195) (SEQ ID NO:16)

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ATTY. DOCKET NO.: REGEN1610-1